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Sigma-1 Receptor Imaging in the Brain

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2014

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Kuzhuppilly Ramakrishnan, N. (2014). *Sigma-1 Receptor Imaging in the Brain: Cerebral sigma-1 receptors and cognition: Small-animal PET studies using 11C-SA4503*. [Thesis fully internal (DIV), University of Groningen]. s.n.

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Population pharmacokinetics of cutamesine in rats using non-linear mixed effects modeling (NONMEM), ^{11}C-SA4503, and microPET

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Submitted

ABSTRACT

Cutamesine (SA4503) is a selective sigma-1 receptor agonist, currently in Phase II clinical trials for depression and post-stroke neurological disturbances. Cutamesine has been found to be effective in several rodent models of amnesia and depression. We used data obtained with carbon-11-labeled cutamesine (^{11}C-SA4503) in rats to develop a population pharmacokinetic (PK) model. Non-linear mixed effects modeling (NONMEM) provides a tool for analyzing repeated measurements data in which the relationship between the explanatory (covariates) and response (PK parameters) variables can be modeled as a single function, allowing the parameters to differ between individuals. This modeling framework can be useful to scale preclinical drug kinetic information to clinical settings.

Methods: MicroPET scans of the brain region of male Wistar Hannover rats (age 1.5-32 months) were made and ^{11}C-SA4503 time-activity curves were obtained for the entire brain. A femoral artery cannula was used for blood sampling, and metabolite-corrected plasma time-activity curves were obtained. Integrated PK models (two-, three- and four-compartment) were explored to describe the plasma and brain time course. Bootstrap resampling (n = 1000) technique was used as a model evaluation tool. The effects of covariates (age, weight and disease condition) on PK parameters were investigated for the final model.

Results: A three-compartment model best described the plasma and brain PK. The three compartments were: a central compartment (plasma) and two brain compartments (free and bound). Population PK parameters (relative standard error) were: central clearance (CL) 17.4 ml/min (13%), central volume of distribution (V_1) 25.3 ml (23%), brain volume of distribution (V_{br}) 84.9 ml (23%), clearance into brain (Q_{in}) 61.8 ml/min (12%), clearance out of brain (Q_{out}) 14.4 ml/min (38%), clearance into bound compartment (Q_{on}) 5.83 ml/min (27%), clearance out of bound compartment (Q_{off}) 1.55 ml/min (4%). Population estimates of the model are in close agreement with the median values of successful bootstrap replicates. **Conclusion:** Population PK modeling can be used successfully to analyze PET data of ^{11}C-labeled cutamesine. This developed model was successfully used to predict the plasma and brain disposition of cutamesine.

Keywords: ^{11}C-SA4503, Cutamesine, Pharmacokinetics, Non-linear mixed effects modeling, MicroPET.

INTRODUCTION

Cutamesine (SA4503) is a selective sigma-1 receptor agonist, currently in Phase II clinical trials for depression and post-stroke neurological disturbances. Cutamesine has been found to be effective in several rodent models of amnesia and depression *(1), (2)*.

Brain penetration, distribution, and binding to the (target) receptor are important aspects for drugs that act through the central nervous system (CNS). Classical pharmacokinetic (PK) studies involving cold drugs will not provide information on target engagement and receptor occupancy. Therefore, proof of concept is difficult. Use of positron emission tomography (PET) data on the contrary will simultaneously show target engagement and allow PK characterization *(3), (4)*. Another advantage of the PET PK methods over conventional PK methods is the availability of brain data. In the conventional PK method, it would be necessary to terminate one animal for each of the brain time points. In the case of human studies obtaining brain data points would be impossible. With PET, a large number of data points can be obtained from the brain of a single animal or subject, allowing more accurate PK parameter estimation.

However, these imaging studies are often low throughput, high cost, and highly labor intensive, consequently, sample sizes (number of subjects) are usually small. In recent years, population-based PK approaches, like non-linear mixed effects modeling (NONMEM), have been used to analyze PET data to overcome problems with sparse data and to maximize the information extracted from the available data *(3), (5)*.

Earlier Kagedal *(5)* and Syvanen et al *(6), (7)* have shown the advantages of using population-based analysis over individual PET analysis in reducing the variability. In conventional PET tracer kinetic modeling, only data from individual animals are modeled. That is, brain and plasma data from each animal is used to arrive at the model parameters for that animal. Therefore, only unexplained variability, the difference between observed radioactivity and the model-fitted radioactivity for that particular observation is accounted for. As a second step, the average and variability of the model parameters are calculated introducing unnecessary variability in the model parameters. In contrast, population-based methods, like NONMEM, models pooled data from all sampled individuals, thereby allowing the quantification of population-typical values as well as two separate kinds of variability. The inter-individual variability, that is explainable, arising from biological processes, and intra-individual variability, that is not explainable, arising from different sources (e.g., measurement error, dosing errors and model misspecification) *(8)*. This methodology may additionally identify the covariates influencing the variability.

A population modeling approach comprises of three main components or models namely, the structural model, statistical model and covariate model *(9)*. The structural model describes the overall trend in the data, making use of the fixed-effects parameters, for example pharmacological clearance and volume of distribution. The statistical component of the model seeks to characterize and quantify any variability in

the population parameters across individuals. Such variability is accounted for using different levels of random effects namely, inter-rat variability (IRV) and within-rat variability (WRV) *(10)*. Finally, the covariate component expresses and characterizes any relationship that may exist between covariates and population model parameters *(11)*. This part seeks to identify and characterize any factors (e.g., age, weight, disease condition) that may cause variability in drug exposure and response.

The purpose of the study was to develop a population-based PK model to describe the time course of carbon-11 labeled cutamesine (^{11}C-SA4503) radioactivity in plasma and brain. For this purpose ^{11}C-SA4503 radioactivity data from different age groups/studies (originally intended to study sigma-1 receptor binding potential and tracer distribution volume) were pooled together to predict pharmacokinetics of ^{11}C-SA4503 by taking into account age-dependent drug disposition. Since, radiolabeled forms of drugs can be used to follow the disposition of the actual drug *(12)*, we predicted PK for the drug cutamesine using the model parameters obtained from the radiolabeled form of the drug and compared it with data obtained from the unlabelled drug.

MATERIALS AND METHODS

Animals

Data obtained from experiments performed in male Wistar Hannover rats aged 1.5, 3, 18, 24 and 32 months (Table 1) were used in this analysis. Animals were either purchased from Harlan (Boxmeer, The Netherlands) or acquired from Semmelweis University (Budapest, Hungary). The rats were housed in Macrolon cages on a layer of wood shavings in a room with constant temperature (21 ± 2°C) and fixed 12-hour light-dark cycle (light phase from 7:00 to 19:00 hours). Food (standard laboratory chow, RMH-B, Hope Farms, The Netherlands) and water were available *ad libitum*. After arrival the rats were acclimatized for at least seven days. Experiments were performed by licensed investigators in compliance with the Law on Animal Experiments in The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen.

PET experiments

PET experiments were performed as described in (Ramakrishnan et al., 2013). Briefly, ^{11}C-SA4503 was prepared by reaction of ^{11}C-methyl iodide with 1-[2-(4-hydroxy-3-methoxy-penthyl)]-4-(3-phenylpropyl)piperazine dihydrochloride (4-O-demethyl SA4503), according to a published method *(13)*. The decay corrected radiochemical yield was ~ 24%, the specific radioactivity was > 100 TBq/mmol at the moment of injection and radiochemical purity > 98%. The ^{11}C-SA4503 solution had pH 6.0 to 7.0.

Before microPET scanning, an arterial cannula was placed in each rat, under isoflurane anesthesia (Pharmachemie BV, The Netherlands, 5% in medical air for induction, 2% for maintenance), for blood sampling and determination of the time

Table 1. Number of animals (n) with weight at the time of PET scanning and injected doses of ^{11}C-SA4503 in the different age groups (median with range is presented)

Age group	1.5 months	3 months	18 months	24 months	32 months
n (scan)	6	8	3	4	3
n (metabolite analysis)	4	3	3	0	5
n (scan, with tumor)	-	-	2	1	2
Body weight (g)	219 (207-228)	294 (272-331)	625 (591-744)	590 (545-643)	585 (491-652)
^{11}C-SA4503 dose in MBq	6.2 (5.3-14.4)	15.05 (11.5-21.4)	39.4 (26.3-45.9)	9.6 (6.8-12.5)	41.8 (24-50.9)
Total number of plasma sample points	91	136	60	45	69
Total number of brain sample points	138	200	115	113	115

course of radioactivity in plasma. From each rat, 15 to 17 arterial blood samples (volume 0.1 to 0.15 ml) were collected after ^{11}C-SA4503 injection (23 +/- 15 MBq) and start of the microPET scan. Twenty five µl of whole blood was reserved and plasma was obtained from the remaining blood by centrifugation. Radioactivity in blood and plasma samples (25 µl) was determined using a calibrated gamma counter (CompuGamma CS 1282, LKB-Wallac, Turku, Finland). Plasma data was corrected for radioactive metabolites based on the age of the rat, as the metabolite formation was found to decrease significantly with age *(14)*. For each of the age groups (except 24 months), 3 to 5 additional rats were used for metabolite analysis. Additional rats were necessary due to the larger volumes of plasma, totaling about 3.5 ml per rat, needed for bio-analysis. Arterial blood samples ranging from 0.4 ml to 1.6 ml were drawn through a femoral artery cannula at 5, 10, 20, 40 and 60 min after injection of tracer. Plasma was obtained by centrifugation and de-proteinated by mixing with one third the volume of 20% trichloroacetic acid in acetonitrile. The mixture was centrifuged for 2 min at 13,000 rpm and the supernatant collected. The parent tracer and metabolites in the supernatant were separated using HPLC, with MicroBondapak C18 (7.8 × 300 mm) column and the mobile phase consisting of a mixture of acetonitrile and 50 mM sodium acetate buffer (pH 6.1) (1/1, v/v) at a flow rate of 3 ml/min. The eluate was collected in 30 sec fractions for 15 min and radioactivity in the samples was measured using a gamma counter.

Two rats were scanned simultaneously in each scan session, using a Siemens/Concorde microPET camera (Focus 220). They were positioned in the camera in transaxial position with their heads and neck in the field of view. First, a transmission scan of 515 sec with a Co-57 point source was obtained for attenuation and scatter correction of 511 keV photons by tissue. Subsequently, the first rat was injected through the penile vein with ^{11}C-SA4503. The emission scan was started with tracer injection of the first rat; whereas the second animal was injected a

few minutes later. A list-mode protocol was used with 90 min acquisition time. Reconstructions were performed using microPET Manager 2.3.3.6; ASIPro 6.3.3.0 (Siemens Solutions, Knoxville, TN). The list-mode data of the emission scans were reframed into a dynamic sequence with frame durations ranging from 30 sec to 720 sec. The data were reconstructed per time frame employing an iterative reconstruction algorithm (OSEM2D with Fourier rebinning, 4 iterations and 16 subsets). The final datasets consisted of 95 slices with a slice thickness of 0.8 mm, and an in-plane image matrix of 128 × 128 pixels. Voxel size was 0.5 × 0.5 × 0.8 mm. The linear resolution at the center of the field-of-view was about 1.5 mm. Data sets were fully corrected for decay, random coincidences, scatter and attenuation.

MicroPET data analysis

The images obtained from the scan were co-registered with an MRI template *(15)* for drawing three-dimensional regions of interest (ROIs) over the whole brain using Inveon Research Workplace software (Siemens Medical Solutions, Knoxville, TN). Time-activity curves (TACs) were calculated and tracer uptake was expressed as Bq/ml, assuming a specific gravity of 1 g/cm^3 for brain tissue and blood plasma (Fig. 1). Individual kinetic analysis was performed by fitting a standard four-parameter, two-tissue compartment model to the dynamic PET data, using metabolite-corrected plasma radioactivity as input function. Software routines for MatLab 7 (The MathWorks, Natick, MA), written by Dr. A.T.M. Willemsen (University Medical Center Groningen, the Netherlands), were used for curve fitting. The blood volume was fixed at 3.6% *(16)* and the rate constants K_1, k_2, k_3, and k_4 estimated from the curve fit.

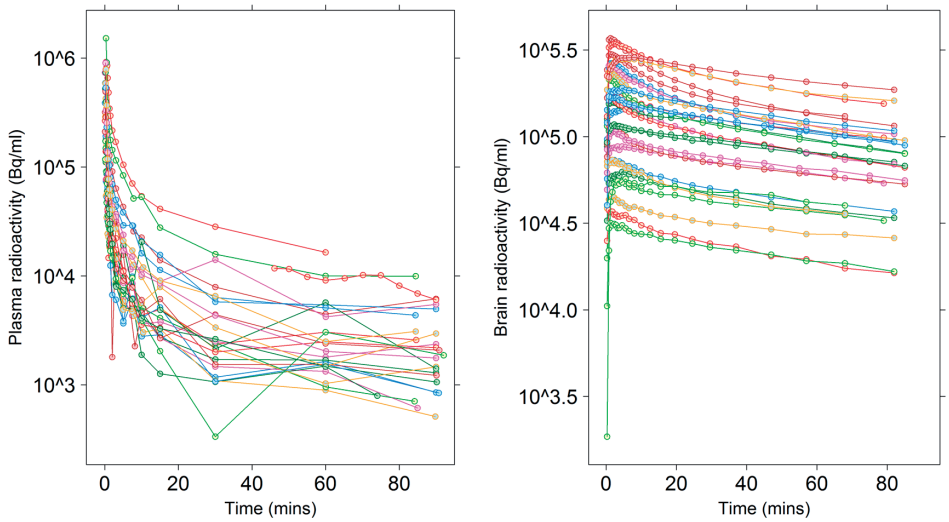

Fig. 1. *Plasma and brain concentration of ^{11}C-SA4503 as a function of time for all rats (plasma PK data: 26 rats, data points: 401; brain PK data: 29 rats, data points: 681).*

Population based-mixed effects modeling for ^{11}C-SA4503

A non-linear mixed effects modeling (NONMEM) approach, performed using the NONMEM VII software *(17)* (ICON Development Solutions, Hanover, MD), was used to describe the time course of plasma and brain PK of ^{11}C-SA4503. Fig. 2 shows the steps that were followed during the model development of the population-based NONMEM analysis. R (version 2.13;www.r-project.org) *(18)(16)* was used for graphical inspection of the results. Natural log-transformed ^{11}C-SA4503 concentrations were used to estimate the plasma and brain PK parameters.

The first-order conditional estimation (FOCE) method in NONMEM with interaction option was used to estimate the model parameters. IRV for the structural model parameters was evaluated using a log-normally distributed model:

$$P_j = PTV \times exp^{(\eta j)} \qquad \textbf{Eq. 1}$$

where PTV represents the population typical value of the parameter and P_j is the value of the parameter for subject j. ηj denotes an subject-specific random effect that distinguishes the value of the jth subject from the PTV. The values of η_j are assumed to be normally distributed with mean zero and variance ω^2. IRV is

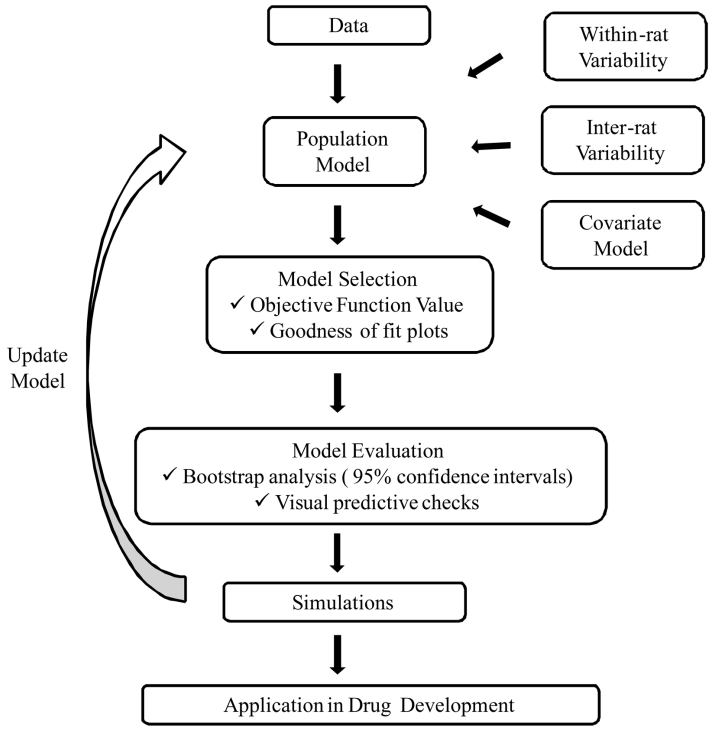

Fig. 2. *Schematic representation of the model development steps that were followed in the population-based non-linear mixed effects modeling analysis.*

expressed as percent coefficient of variation (% CV). The WRV describes the error terms, which remain unexplained and refers to, for example, dosing inaccuracies, analytical errors, or structural model misspecifications. Different types of WRV residual error models (e.g., proportional, additive) were tested separately for plasma and brain. A proportional error model was used to describe WRV in the plasma and brain concentration as shown in the following equation:

$$ln(y_{ij}) = ln(\hat{y}_{ij}) + \varepsilon_{ij} \quad \textbf{Eq. 2}$$

where y_{ij} is the j^{th} observation in the i^{th} animal, $\hat{y}_{ij}$ is the corresponding model prediction, and ε_{ij} is a normally distributed random error with a mean of zero and a variance of σ^2.

Model selection

Model selection was based on the precision of parameter estimates (as relative standard errors; RSE), the objective function value (OFV) and the goodness-of-fit (GOF) plots. The RSE should ideally be less than 30% for each PK parameter for the model to be selected, although values up to 50% are acceptable. A reduction of OFV by 3.84 (corresponding to a *P* value of 0.05) is required, for each additional structural parameter or IRV, to consider a model with additional parameters as superior *(19)*. GOF was assessed graphically by evaluation of the agreement between observed and predicted ^{11}C-SA4503 concentrations, the range of conditional weighted residuals, and uniformity of the distribution of conditional weighted residuals about zero *(20)* across the range of the predicted concentrations.

To describe the time course of combined plasma and brain levels, three model structures were investigated as shown in Fig. 3; a) a two-compartment model with a central compartment for plasma and a peripheral compartment for brain, b) a three-compartment model with a central compartment for plasma and two brain compartments (free and bound drug), c) a four-compartment model with a central plasma compartment, two brain compartments and a peripheral compartment. Based on RSE, OFV and GOF plots, the best performing model was chosen, as shown in the box of Fig. 3.

The final structural PK model (Fig. 3b), consisted of a three-compartment model. The concentration-time profile of ^{11}C-SA4503 in the plasma compartment can be described by equation 3. From the plasma compartment, ^{11}C-SA4503 diffuses across the blood-brain barrier into the brain (brain free, equation 4), where it can bind to the sigma-1 receptors (brain bound, equation 5). In order to improve the stability of the model, a model with a common Vbr (Vbr1 + Vbr2) parameter was used, which corresponds to total brain volume (brain free + brain bound). Total ^{11}C-SA4503 concentration in brain is derived from equation 6.

Differential equations: This final model was implemented by user-defined differential equations using the ADVAN 9 subroutine in NONMEM.

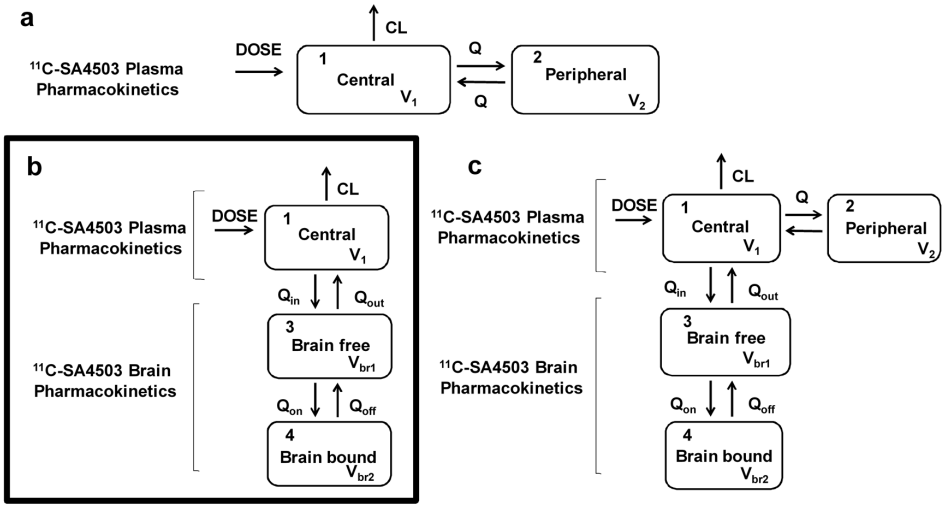

Fig. 3. *The population pharmacokinetic model structures. V_1, V_2, V_{br1} and V_{br2} are pharmacological volumes of distribution in central, peripheral, brain free and brain bound concentration compartments, respectively. CL (total body clearance), Q (bi-directional clearance between central and peripheral compartments), Q_{in} (clearance into brain free compartment), Q_{out} (clearance out of the brain free compartment), Q_{on} (clearance into bound compartment), and Q_{off} (clearance out of bound compartment). The model that best described the data is model b (box).*

Plasma

dA1/dt(1) = K21*A(2)-KE*A(1)-K12*A(1) **Eq. 3**

Where, Cplasma = A1/V1 ; KE=CL/V1 ; K12=Q/V1

Brain

dA2/dt (2) = K12*A(1)+K32*A(3)-K21*A(2)-K23*A(2) **Eq. 4**

dA3/dt (3) = K23*A(2)-K32*A(3) **Eq. 5**

Total concentration in brain was quantified using equation 6

Cbrain = (A2+A3)/(Vbr1 + Vbr2) **Eq. 6**

Influence of rat weight, age, and presence of pituitary tumor were evaluated as covariates to account for the variability in the PK model parameters. We also tested age as a binary covariate (aged rats- 18, 24 and 32 months vs. young rats- 1.5 and 3 months) on brain PK parameters. Covariate analysis was performed in NONMEM using PsN with a step-wise forward additive approach followed by a step-wise backward elimination approach with a *P* value of 0.05 and 0.01, respectively *(21)*. Uncorrelated covariates were included in the model using different functional forms like linear, power and exponential functions.

Model evaluation

The bootstrap analysis was performed to check the stability of the PK model. In this analysis, bootstrap replicates are generated by random sampling from the original data set with replacement. Summary non-parametric model parameters (bootstrap median and 95% confidence intervals) were calculated and compared with observed parameter values.

In addition, a predictive check was performed to determine whether the final integrated model (plasma and brain PK model) provides an adequate description of tracer disposition. For this purpose, one thousand Monte-Carlo simulations were performed utilizing the final model parameter estimates to compare the distribution of the simulated and the observed plasma and brain PK data. Observed data was overlaid on 95% prediction intervals arising from this simulation. This model may be used to simulate data that are suitable for direct comparison with the observed data as described in the following section.

Application of tracer PK model to predict cutamesine PK

The developed tracer PK model was subsequently used for predicting the PK and absolute bioavailability of cutamesine at 1.0 mg/kg dose in 3 month old rats. This dose, administered intra-peritonealy (i.p), was used in behavioral tests for learning and memory and receptor occupancy studies (unpublished data). At the end of the behavioral studies, the rats were allowed a wash out period of at least two weeks before the pharmacokinetic study. They were anesthetized as for the scan, cutamesine was administered under isoflurane anesthesia and blood sampled through a cannula inserted in the femoral artery (cannulated as for the scan). Additional plasma samples (n = 16) collected between 75 to 180 min post-dosing, during a receptor occupancy study scan (with similar anesthesia and cannulation procedure), were also included in the present study. Plasma area under the curve after i.p administration (AUC_{ip} = 197 ng×h/mL), of cutamesine exposure obtained from conventional PK analysis was used for calculating bioavailability (equation 7).

$$F_{ip}*Dose = CL*AUC_{ip}. \quad \textbf{Eq 7}$$

RESULTS

PET data

Fig. 1 depicts time profiles of ^{11}C-SA4503 in plasma and brain. The tracer was rapidly cleared from the plasma and reached a plateau within 5 min. In the brain, a peak was observed within 10 min and was followed by a slow washout.

Population based-mixed effects modeling for ^{11}C-SA4503

Model b (Fig. 3), a three-compartment model performed the best based on OFV (model a: -715; model b: -3325; model c: -3298). The three compartments were a central compartment (plasma), and two brain compartments (free and bound). Population PK parameters (relative standard error) were: central clearance (CL) 17.4 ml/min (13%), central volume of distribution (V_1) 25.3 ml (23%), brain volume of distribution (V_{br}) 84.9 ml (23%), clearance into brain (Q_{in}) 61.8 ml/min (12%), clearance out of brain (Q_{out}) 14.4 ml/min (38%), clearance into bound compartment (Q_{on}) 5.83 ml/min (27%), clearance out of bound compartment (Q_{off}) 1.55 ml/min (4%). 95% confidence intervals of above PK parameters obtained from nonparametric bootstrap analysis are shown in Table 3. Relationship between NONMEM population parameter estimates and the PET parameters from a two-tissue compartment model are also presented in Table 3. Generally, parameter estimates were similar for individual and population modeling approaches.

Model GOF plots for the final population PK model including both brain and plasma ^{11}C-SA4503 concentrations are shown in Fig. 4. Most of the data points are randomly distributed around the line of identity which indicates that the model describes the time course adequately. In addition, randomly selected individual plots showing the observed vs. predicted ^{11}C-SA4503 radioactivity is displayed in Fig. 5. Individual profiles are adequately described by the model after accounting for variability.

Only one covariate, age as a continuous variable, was found to significantly (P <0.01) influence the final PK model ($Q_{in;}$ reduction of 1.6% for a unit of month from median age of 18 months). We also observed age as a binary covariate (aged vs. young rats) on brain volume (Vbr), but the parameter estimate was imprecise. Though other covariates (weight and presence of tumor) resulted in a significant drop of OFV they resulted in high %RSE in covariate-parameter estimates.

Model evaluation

Population parameter estimates from the PK model are in good agreement with the median values of successful bootstrap replicates (Table 3). Simulation-based visual predictive check plots are shown in Fig. 6. More than 80% of the observed

Table 2. Number of samples collected at each time point from 3 month old rats for conventional PK study after intraperitoneal administration of 1 mg/kg cutamesine

Time (min)	1	2	5	15	30	45	60	75	90	120	180	300	1440
Plasma samples	1	1	8	8	7	5	5	3	3	1	-	2	2
Brain (n = 13) samples	-	1	-	1	2	-	2	-	2	1*	-	2	2
Plasma samples (from receptor occupancy study)	-	-	-	-	-	-	-		16			-	-

* Data lost during sample preparation

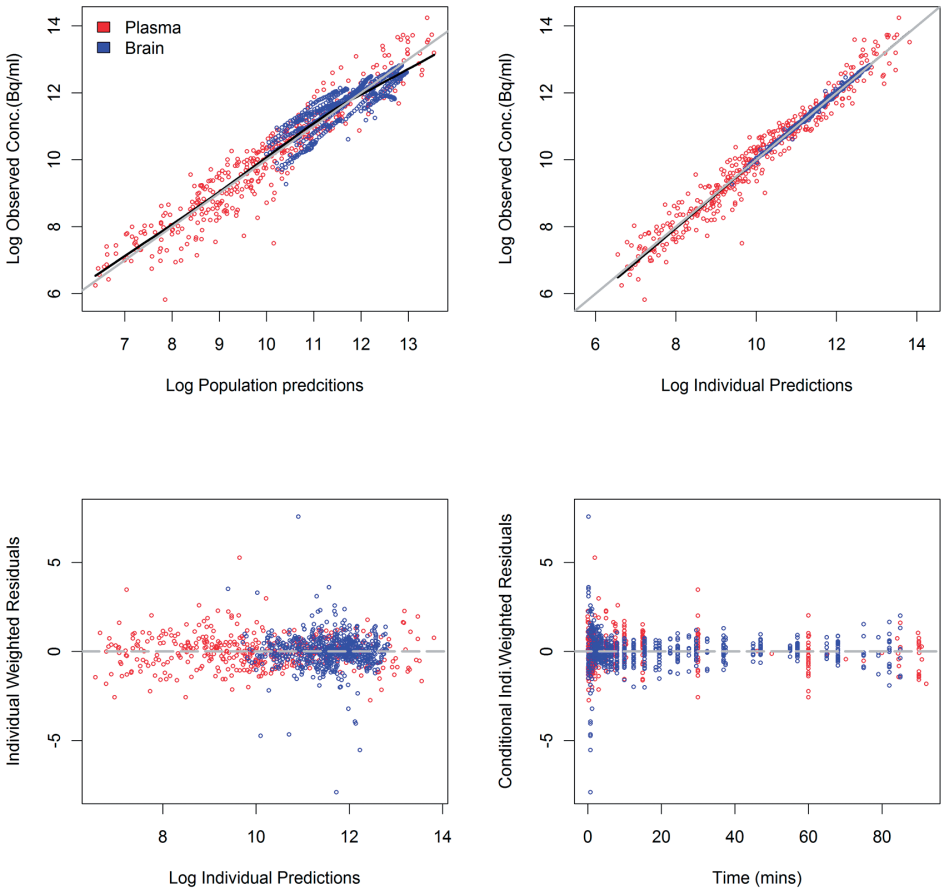

Fig. 4. *Model diagnostic plots (GOF plots) for the final population pharmacokinetic model including both brain and plasma ^{11}C-SA4503 concentrations. All dots represent individual data points. Observed versus population and observed versus individual predictions are shown in the upper panels. Most of the data points are randomly distributed around the line of identity (grey) which indicates that the model describes the concentrations adequately. Absolute individual weighted residuals versus individual predictions and conditional weighted residuals versus time are shown in the lower panels. Most residuals are scattered around zero, indicating suitability of the model for further model evaluation approaches.*

data points fell within 95% prediction intervals (shaded area), indicating the adequacy of the model to reproduce the observed data.

Application of tracer PK model to predict the cold SA4503 (cutamesine) PK

The developed tracer PK model was subsequently used for predicting the PK of cutamesine at a 1.0 mg/kg dose in 3 month old rats. The bioavailability (F%) following intra-peritoneal dosing of cutamesine to 3 months old rats was found

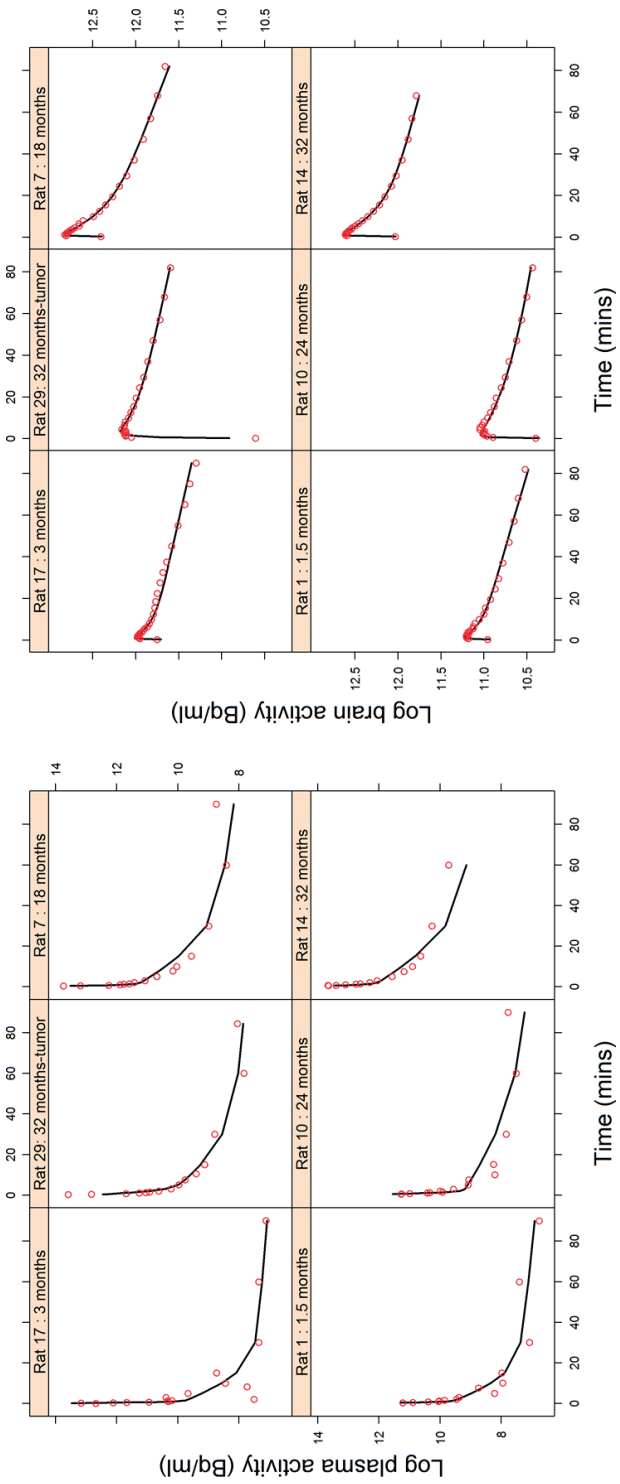

Fig. 5. *Individual plots of plasma and brain ^{11}C-SA4503 concentrations for the final model. The open circles represent observed measurements and the lines are model predictions.*

Table 3. Population pharmacokinetic (PK) parameter estimates from the original data set for the final model and 95% confidence interval (CI) resulting from 1,000 bootstrap replicates

PK Parameter	Mean (95% CI*)	IRV as % CV (95% CI*)
Plasma pharmacokinetics		
V_1 (ml)	25.3 (19.8-32.3)	52 (36-64)
CL (ml/min)	17.4 (14.9-19.6)	33 (24-41)
WRV for plasma (CV %)	40 (34-49)	-
Brain pharmacokinetics		
$V_{br1} = V_{br2}$ (ml)	84.9 (68.7-99.4)	37 (25-46)
Q_{in} (ml/min)	61.8 (52.6-76.5)	18 (4-34)
Q_{out} (ml/min)	14.4 (11.0-21.4)	35 (20-62)
Q_{on} (ml/min)	5.83 (3.71-8.74)	41(17-70)
Q_{off} (ml/min)	1.55 (1.20-2.01)	NE
WRV for Brain (CV %)	4.1 (2-5)	-
Relationship between non-linear mixed effects modeling population parameter estimates and the PET parameters from two-tissue compartment model		
Parameter (SE)	**PET approach**	**Population-based approach**
K_1 (ml ml^{-1} min^{-1})	1.45 (0.40)	1.46 (0.38)
k_2 (min^{-1})	0.64 (0.25)	0.34 (0.29)
k_3 (min^{-1})	0.165 (0.025)	0.137 (0.08)
k_4 (min^{-1})	0.030 (0.0025)	0.037 (0.0035)
Rate constants were calculated based on the model estimates shown above:		
$K_1 = Q_{in}/V_1(V_1/V_{br})$		
$k_2 = Q_{out}/V_{br1}$		
$k_3 = Q_{on}/V_{br1}$		
$k_4 = Q_{off}/V_{br2}$		

NE: not estimated

to be about 60% using equation 7. The model, based on the radiotracer, predicted the plasma time course of cutamesine reasonably well after correction for bioavailability. However, the brain exposure was under predicted for cutamesine compared to the tracer. We observed approximately 7 times higher brain to plasma ratio for ^{11}C-SA4503 compared to cutamesine. Following the correction for the difference in brain-to-plasma ratio and bioavailability, the model predictions were in reasonably good agreement with the observed brain PK data. The overlay of model predictions and real observed data for 1 mg/kg intra-peritoneal administration is shown in Fig 7.

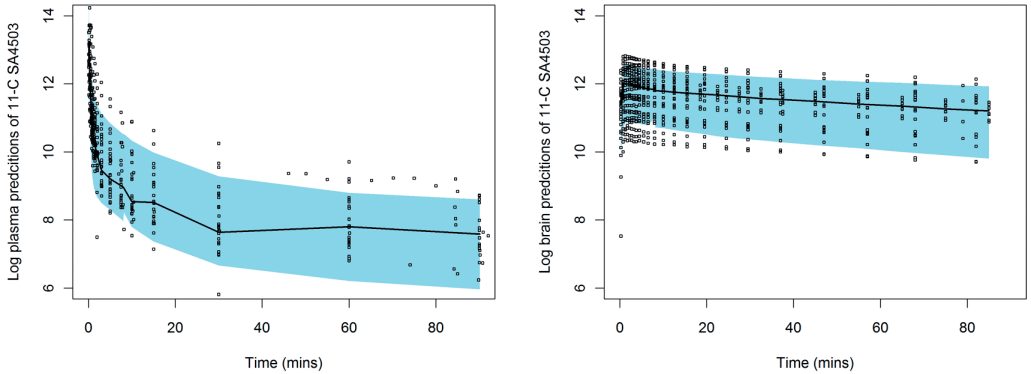

Fig. 6. *A visual predictive check plot of the ^{11}C-SA4503 final pharmacokinetic model. The shaded areas represent the 95% confidence intervals of the simulated data and the black solid line represents the median of the observed data. Dots represent individual observed data points.*

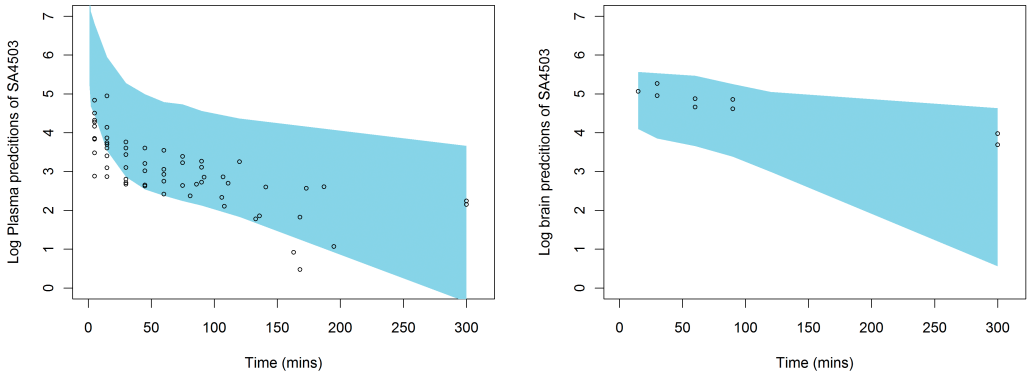

Fig. 7. *Predicted pharmacokinetic exposure of cold SA4503 (cutamesine), in plasma and brain compartments, at its therapeutic dose of 1 mg/kg. The shaded areas represent the 95% confidence intervals of the simulated data (from PET study) and the dots represent individual observed data points (from conventional PK study).*

DISCUSSION

Cutamesine (SA4503) is a selective sigma-1 receptor agonist, currently in Phase II clinical trials for depression and post-stroke neurological disturbances [http://www.clinicaltrials.gov/ct2/results?term=SA4503&Search=Search]*(22)(20)*. In this paper we presented the development of a population-based PK model to describe the time course of ^{11}C-SA4503 radioactivity in plasma and in brain. Additionally, we also showed its utility in predicting the behavior of the unlabelled drug, cutamesine. The radiotracer model adequately predicted the plasma exposure after correcting for bioavailability, while an additional correction for brain-to-plasma ratio was required for the brain. We speculate that protein binding, active transport or saturation of binding at receptors may have contributed to this difference since the model was developed at tracer concentrations, whereas the drug at 1 mg/kg is in the pharmacological range. At the 1 mg/kg dose, over 90% of sigma-1 receptors would be occupied (reported elsewhere) and the ratio of bound/free drug is reduced, whereas at tracer concentrations, bound/free approaches Bmax/Kd.

In this analysis, all plasma and brain data were simultaneously fitted and potential differences in model parameters from these two regions are considered including a WRV term separately for plasma and brain compartments. This is in contrast to tracer kinetic modeling, where it is not possible to separate the variability coming from the plasma and brain. For example, from our results (Table 3) it can be seen that plasma data accounts for a much higher unexplained variability (40% CV) than brain (4.1% CV). Since unexplained variability can generally be assumed to represent a combination of measurement errors, dosing errors and model misspecification, we can conclude that brain TACs derived from PET is more accurate and robust compared to the plasma TACs obtained from gamma counter which requires much more handling of the samples. Such separation and explanation of variability would not be possible with conventional tracer kinetic modeling.

NONMEM uses population data and allows pooling of data from different studies, centers, species etc to improve parameter precision and thereby to increase the confidence of dose or exposure (in plasma or brain) versus response relationship. Since brain drug concentrations are seldom measured in humans, it is not possible to study the drug exposure in human brain and its relationship with effects. Understanding the plasma and brain exposure-response relationship of new drugs pre-clinically prior to testing in humans would be of great value in the clinical setting as it would help in choosing the dose and dosing schedule *(23)*. This could be achieved using modeling tools which allow translating this relationship from one species to another species using allometric approaches. Using appropriate scaling factors and human specific information on PK and receptor binding, one could estimate pharmacokinetic-pharmacodynamic parameter in humans using allometric scaling: for e.g., PK parameter in humans = PK parameter in Rat* (Weight human/Weight Rat)^^{b}; (b = is exponent. e.g., for CL or Vd, the commonly used exponent for CL = 0.75 and for Vd = 1). Moreover,

model-based approaches can accommodate the diverse drug-related features (e.g., physico-chemical properties, *in vitro* efficacy and safety profile and metabolic properties) and the system-related features (e.g., receptor density, transduction process, and disease progression) which can increase the efficiency in drug development of CNS drugs *(24), (25)*. Population-based approaches therefore allow maximizing the extraction of information from available data.

The identification of covariates (e.g., age, weight) that explain variability is an important objective of any population modeling evaluation. Covariate modeling helps to answer questions like "how much does a young rat differ in exposure from old rats?" In our covariate analysis, age as a continuous variable had an influence on Q_{in}. We also observed age as a binary covariate on brain volume. Based on these results we anticipate that brain drug exposure might be reduced with aging and higher doses of the drug may be necessary in aged rats. The reduced brain volume may be due to the reduced transport of the drug into the brain with age. More rats in each age group could allow identification of other significant covariate-parameter relationships. Combined analysis of the population-based PK with PET data allows prediction of not only the time course of drug but also helps to quantify the exposure required for efficacy and safety.

CONCLUSIONS

We characterized the PK of the drug cutamesine using data obtained from ^{11}C-SA4503 microPET studies in rats. A population-based PK model was developed to provide robust model parameter estimates which were subsequently used to predict the plasma and brain disposition of cutamesine. It was also evident from our analysis that the population-based approach showed generally comparable parameter estimates in comparison with individual tracer kinetic approach method. In addition, population-based modeling approach helped to quantify the variability within and between rats.

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